understood. A key open question is whether–and how, if so–the system deals with impediments to motion, to avoid or minimize potential traffic jams. Because more motors can exert a larger force, and drive the cargo further, one might use more motors on average to address challenges to motion, but this solution comes at the cost of increased average energy consumption. Instead, we discover a cargo-level adaptation: when challenged with opposition to motion, cargos dynamically increase their sustained force production capability to 'power through' the obstacle. This adaptation improves minus-end force production, and improves ability to overcome obstacles between three and four-fold.

44-Subg
Stochastic Simulations of Cellular Processes: From Single Cells to Colonies
Zaida Luthey-Schulten.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
No abstract.

45-Subg
Transcription Against Supercoiling
Sunney Xie.
Chem/Chem Biol, Harvard University, Cambridge, MA, USA.
No abstract.

Subgroup: Biological Fluorescence

46-Subg
Probing Spatiotemporal Regulation of Signal Transduction in Living Cells
Jin Zhang, Ph.D.
Pharmacology, Johns Hopkins University, Baltimore, MD, USA.
Jin Zhang, Departments of Pharmacology & Molecular Sciences, Neuroscience and Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205
The complexity and specificity of many forms of signal transduction are widely suspected to require spatial microcompartmentation and dynamic modulation of the activities of signaling molecules, such as protein kinases, phosphatases and second messengers. I will present studies where we combined genetically encoded fluorescent biosensors, targeted biochemical perturbations, mathematic modeling and superresolution imaging to probe the spatiotemporal regulation of signaling molecules such as cAMP, Protein Kinase A, calcineurin and Protein Kinase B.

47-Subg
In Vivo Deep Tissue Multiphoton Microscopy
Chris Xu.
Cornell University, Ithaca, NY, USA.
I will present our latest progress in multiphoton fluorescence microscopy.

48-Subg
Fluorescence Polarization and Fluctuation Analysis Reveals Changes in Camkii Holoenzyme Organization with Activation and Subsequent T-Site Interactions
Steven S. Vogel, Ph.D.
LMP, NIAAA/NIH, Rockville, MD, USA.
No abstract.

49-Subg
Quantitativesuper-Resolution Imaging of Biological Processes with High Spatiotemporalresoltuion
Melike Lakadamyali, PhD.
Molecular and Cellular Biology, ICFO-Institute of Photonic Sciences, Castelldefels, Spain.
In the last decade, far field optical microscopy has undergone a revolution, in which a number of methods have been developed that break the diffraction limit of spatial resolution. These methods allow imaging biological structures and protein organization at the nanometer length scales, opening the door for exciting new discoveries in biology. However, the super-resolution methods have certain limitations. One such limitation concerns live cell applications and the trade-off between spatial and temporal resolution, which makes it challenging to achieve high spatiotemporal resolution simultaneously. We have recently developed an all-optical correlative imaging platform to circumvent this problem and applied it to study how motor proteins overcome obstacles and roadblocks to move cargo from one place to other in the complex cellular environment.
An additional complication in super-resolution microscopy is the ability to quantify the images to count proteins and extract information about protein stoichiometry. This challenge stems from the complex biophysics of fluorescent dyes and fluorescent proteins. We have also developed ways to characterize these photophysical properties in order to quantify super-resolution images.
In this talk, I will explain the progress we have made in both correlated live-cell and super-resolution imaging as well as quantitative super-resolution imaging. I will also give examples of biological applications along both lines.

50-Subg
Imaging Fluorescence Correlation Spectroscopy Measures Dynamics and Structure in Live Samples
Thorsten Wohland.
Chemistry and Center for Bioimaging Sciences, National University of Singapore, Singapore, Singapore.
Fluorescence Correlation Spectroscopy (FCS) has been developed more than 40 years ago and has evolved into a major tool in the biophysical sciences for the quantitative measurement of biomolecular dynamics and interactions. In the past FCS was mainly used as a confocal, single spot measurement technique. Imaging FCS, i.e. the recording of FCS measurements at all pixels in an image, became feasible only recently with the advent of fast, sensitive array detectors and the development of new illumination modes in microscopy, in particular light sheet microscopy. Despite the fact that the time resolution of imaging FCS is lower than for single spot measurements, imaging FCS has several advantages. The most important being i) that it contains much more information since spatial and temporal correlations can be evaluated between any pixels or group of pixels in the image, and ii) the illumination modes (TIRFM or SPIM) expose the sample to much lower light doses and allow more measurements per sample.
We demonstrate the capabilities of imaging FCS using total internal reflection (TIR) and single plane illumination microscopy (SPIM) as illumination modes. We investigate the membrane active human Islet Amyloid Polypeptide (hIAPP) on cell membranes using imaging TIR-FCS, FCS diffusion laws, and time-lapse FCS videos, to elucidate the membrane action of monomeric hIAPP. With the aid of the FCS diffusion laws, Imaging FCS can access information about sample structure even below the diffraction limit. And light sheet illumination allows single cell observations over up to one hour (total effective illumination time ~10 minutes) without visible cell damage. Second, we use SPIM-FCCS to demonstrate the measurement of interactions of biomolecules in vitro and in live cells. These experiments show that Imaging FCS provides quantitative images of biological samples under physiological conditions.

Subgroup: Mechanobiology

51-Subg
In Vitro Contraction of Cytokinetic Ring Depends on Myosin II but not on Actin Dynamics
Mithilesh Mishra[1,†], Jun Kashiwazaki[2,†], Tomoko Takagi[2], Ramanujam Srinivasan[3], Yinyi Huang[3], **Mohan K. Balasubramanian**[1,3,4,*], Issei Mabuchi[2,*].
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Cytokinesis in many eukaryotes involves the contraction of an actomyosin-based contractile ring (CR)1,2. However, the detailed mechanism of CR contraction is not fully understood. Here, we establish for the first time an experimental system to study contraction of the CR to completion in vitro. We show that CR of permeabilised fission yeast cells undergo rapid contraction in an adenosine triphosphate (ATP) and myosin-II dependent manner in the absence of other cytoplasmic constituents. Surprisingly, neither actin polymerisation nor its disassembly is required for contraction of CR although addition of exogenous actin cross-linking proteins block CR contraction. Using CRs generated from fission yeast cytokinesis mutants, we show that not all proteins required for assembly of the ring are required for its contraction in vitro. Our work provides the beginnings in the definition of a minimal contraction-competent cytokinetic ring apparatus.